

Dedy DARNAEDI^{*,**}, Masahiro KATO^{*} & Kunio IWATSUKI^{*}:

**A cytotaxonomic study of *Dryopteris sparsa* and
closely related species (Dryopteridaceae)**

デディ ダルナエディ^{*,**}・加藤雅啓^{*}・岩槻邦男^{*}: ナガバノ
イタチンダおよび近縁種の細胞分類学的研究

Dryopteris sparsa (Ham. ex D. Don) O. Kuntze has been regarded as a most polymorphic species with a wide geographical range from the Himalayas and Sri Lanka to southern Japan and New Guinea. Using mainly comparative morphology, Darnaedi et al. (1989) recognized five species closely related to *D. sparsa*. They include two Taiwanese species, *D. melanocarpa* Hayata and *D. caccina* Tagawa, which were treated as conspecific with *D. sparsa* by DeVol & Kuo (1975) and with *D. yakusilvicola* Kurata by Fraser-Jenkins (1986), respectively. The other three are *D. rheophila* Mitsuta ex Darnaedi et al., *D. angustipalea* Darnaedi et al., and *D. indonesiana* Darnaedi et al. *Dryopteris rheophila* is a rheophyte known from northeastern Thailand, while *D. angustipalea* is endemic to Seram Island, the Moluccas, and *D. indonesiana* is distributed in scattered localities in Indonesia. In addition, several interspecific hybrids involving *D. sparsa* have been recorded: *D. rheophila* × *D. sparsa* in northeastern Thailand, *D. sabaei* × *D. sparsa* var. *sparsa* and *D. sabaei* × *D. sparsa* var. *ryukyuensis* in Yakushima Island (Darnaedi 1989), and *D.* × *rarissima* Kurata and *D.* × *yamashitae* Kurata in Kyushu (cf. Nakaïke 1975).

Previous cytological observations have shown that *D. sparsa* includes three cytotypes, i.e., diploid (sexual), triploid (agamosporous), and tetraploid (sexual) (Darnaedi & Iwatsuki 1987, Gibby 1985 as *D. viridescens*, Hirabayashi 1974, Kurita 1966, Manton 1955, Manton & Sledge 1954, Mehra 1961). However, cytological information of the species in Southeast Asia, the distribution center of the species, is lacking, except for the Malay Peninsula (Manton 1955).

Cytological data provides evidence for reproductive mode, if both somatic

* Botanical Gardens, Faculty of Science, University of Tokyo, 3-7-1 Hakusan, Bunkyo-ku, Tokyo 112. 東京大学 理学部附属植物園.

** Present address: Herbarium Bogoriense, Pusat Penelitian dan Pengembangan Biologi-LIPI, Jl. Raya Juanda 22-24, Bogor, Indonesia. ボゴール植物標本館 (インドネシア).

and gametic chromosome numbers are counted for single individuals (Manton 1950, Lovis 1977). Although the reproductive mode of *D. sparsa* has been partly presumed by using chromosome pairing data supplemented by the number of spores (Hirabayashi 1974), our understanding of the reproductive mode of the members of this species complex is far from complete.

In this study, we examined the cytology and reproductive mode of the *D. sparsa* complex.

Materials and methods Materials used in this study are listed in Tab. 1. They include *D. sparsa* (53 plants), *D. melanocarpa* (10), *D. angustipalea* (2), *D. rheophila* (12), *D. sabaei* × *D. sparsa* var. *sparsa* (1), *D. sabaei* × *D. sparsa* var. *ryukyuensis* (2), and *D. sparsa* × *D. rheophila* (2). They were cultivated in a greenhouse of the Botanical Gardens, Faculty of Science, University of Tokyo. *Dryopteris caccina* and *D. indonesiana* were not examined cytologically, due to lack of living material.

For gametic chromosome observations, portions of young fertile leaves were fixed in glacial acetic acid-absolute ethanol (1:3) solution, and kept at room temperature for 24 hr. Chromosome counting was made on spore mother cells at meiosis, with the ordinary aceto-carmin squash method (Manton 1950). For somatic chromosome observations, active root tips were pretreated with 0.002 M 8-hydroxyquinoline solution, and kept in darkness at 20°C for 3 hr. The root tips, from which root caps were separated, were fixed in 45% acetic acid for 10 min, macerated in 1 N HCl solution at 60°C for 2-3 min, and then squashed in 2% aceto-orcein solution.

To help determine reproductive mode, the number of spores in one sporangium was also counted for all voucher specimens for the cytological observations and 155 additional herbarium specimens. Sixty-four-spored plants are assumed to reproduce sexually, and 32-spored plants to reproduce agamosporously (Manton 1950, Lovis 1977, Walker 1979).

Voucher specimens for the cytological study are deposited in the Herbarium, Faculty of Science, University of Tokyo (TI).

Results The results of our observations are shown in Tab. 1. *Dryopteris sparsa* included four cyto-reproductive types: sexual diploid ($n=41$ and $2n=82$) (Fig. 1A, B) and tetraploid ($n=82$ and/or $2n=164$) (Fig. 1C, D), and agamosporous diploid ($'n'=82$ and $2n=82$) (Fig. 1E, F) and triploid ($'n'=123$ and/or $2n=123$) (Fig. 1G, H). The sexual diploid as determined by both somatic and

Tab. 1. Materials, localities and voucher specimens of *D. sparsa* and its closely related species, and their chromosome numbers, ploidy level and reproductive mode.

| Species & hybrid (number of materials), locality, specimen | Chromosome number (n/2n) | Ploidy & reproduction |
|---|--------------------------|-----------------------|
| <i>D. sparsa</i> (53) | | |
| Mt. Salak, West Java; D. Salak-4 | 41/82 | 2× sexual |
| Phu Kradung, Northeast Thailand; TY. 50A, TY. 50C | 82/- | 4× sexual |
| Phu Kradung, Northeast Thailand; TY. 54A, TY. 54C, TY. 54D | 82/164 | 4× sexual |
| Bali; D. Bali-3, D. Bali-7 | -/164 | 4× sexual |
| Bali; D. Bali-4, D. Bali-5 | 82/164 | 4× sexual |
| Purwodadi, East Java; Pur. 1, Pur. 16, Pur. 18, Pur. 24/5, Pur. 25/11 | -/164 | 4× sexual |
| Cibodas, West Java; Cib. 2, Cib. 3, Cib. 4, | -/164 | 4× sexual |
| Cibodas, West Java; Cib. 86-129 | 82/164 | 4× sexual |
| Cibodas, West Java; Cib. 86-130 | 82/- | 4× sexual |
| Seram, Moluccas; C-6879, C-7405 | 82/164 | 4× sexual |
| Seram, Moluccas; C-3548 | -/164 | 4× sexual |
| Malaya; MK. Mala-2, MK. Mala-3 | 82/164 | 4× sexual |
| Ambo-rindo, Yakushima; D. 77, D. 78 | -/164 | 4× sexual |
| Mt. Mocchomudake, Yakushima; Mo-1, Mo-5, Mo-10 | -/164 | 4× sexual |
| Nagata River, Yakushima; Na-1, Na-4 | 82/164 | 4× sexual |
| Miyanoura, Yakushima; Miya-21, Miya-22, Miya-23 | 82/- | 4× sexual |
| Hanaage River, Yakushima; Han. 2, Han. 7 | -/164 | 4× sexual |
| Iriomote, Ryukyu Isls.; TY. 11 | 82/- | 4× sexual |
| Ambo-rindo, Yakushima; D. Ambo IV-4, TY. 600-8 | 82/82 | 2× agamosporous |
| Ambo-rindo, Yakushima; TY. 600-2, TY. 600-13 | 123/- | 3× agamosporous |
| Kusugawa, Yakushima; Ku. 1, Ku. 3, Ku. 5 | 123/- | 3× agamosporous |
| Nakabase River, Yakushima; D. 721-2(3), Naka-2 | 123/- | 3× agamosporous |
| Osaki River, Yakushima; Os. 3, Os. 7 | 123/- | 3× agamosporous |
| Osaki River, Yakushima; Os. 8 | -/123 | 3× agamosporous |
| Owase, Mie Pref.; D. Mie-15 | 123/123 | 3× agamosporous |

Tab. 1. (continued)

| Species & hybrid (number of materials), locality, specimen | Chromosome number (n/2n) | Ploidy & reproduction |
|---|--------------------------|-----------------------|
| Cibodas, West Java; D. MK-III, D. Cib. 86-13 | 123/123 | 3× agamosporous |
| <i>D. melanocarpa</i> (10) | | |
| Mt. Arisan, Taiwan; JM & TK. 7 | 41/- | 2× sexual |
| Mt. Arisan, Taiwan; JM & TK. 1, 2, 11, 12, 13, 14, TH & SP. 1, Kuo. 4 | -/82 | 2× sexual |
| Mt. Arisan, Taiwan; JM & TK. 5 | -/123 | 3× agamosporous |
| <i>D. angustipalea</i> (2) | | |
| Seram, Moluccas; C-6851, C-13213 | 41/82 | 2× sexual |
| <i>D. rheophila</i> (12) | | |
| Phu Kieo, Northeast Thailand; TY. 49D, TY. 49X | 82/- | 4× sexual |
| Phu Kradung, Northeast Thailand; TY. 48A, TY. 48X, TY. 49Y, TY. 52 | -/164 | 4× sexual |
| Phu Kradung, Northeast Thailand; TY. 48Z, TY. 52A, TY. 52I, TY. 52X, TY. 52Y, TY. 52Z | 82/164 | 4× sexual |
| <i>D. sabaei</i> × <i>D. sparsa</i> var. <i>sparsa</i> (1) | | |
| Ambo-rindo, Yakushima; D. Ambo III-13 | meiosis irregular/123 | 3× sterile |
| <i>D. sabaei</i> × <i>D. sparsa</i> var. <i>ryukyuensis</i> (2) | | |
| Mt. Mocchomodake, Yakushima; D. 40aH, D. 44a | meiosis irregular/123 | 3× sterile |
| <i>D. rheophila</i> × <i>D. sparsa</i> (2) | | |
| Phu Kradung, Northeast Thailand; TY. 50B, TY. 54L | meiosis irregular/164 | 4× sterile |

gametic counts was from Mt. Salak, western Java; the sexual tetraploids from northeastern Thailand, Malay Peninsula, Java, Bali, Seram, and southern Japan; the agamosporous diploid from Yakushima Island; and agamosporous triploids from southern Japan and Cibodas, western Java. The agamosporous diploid is known from only one locality in Yakushima Island.

Dryopteris melanocarpa had two types, a sexual diploid ($n=41$ or $2n=82$)

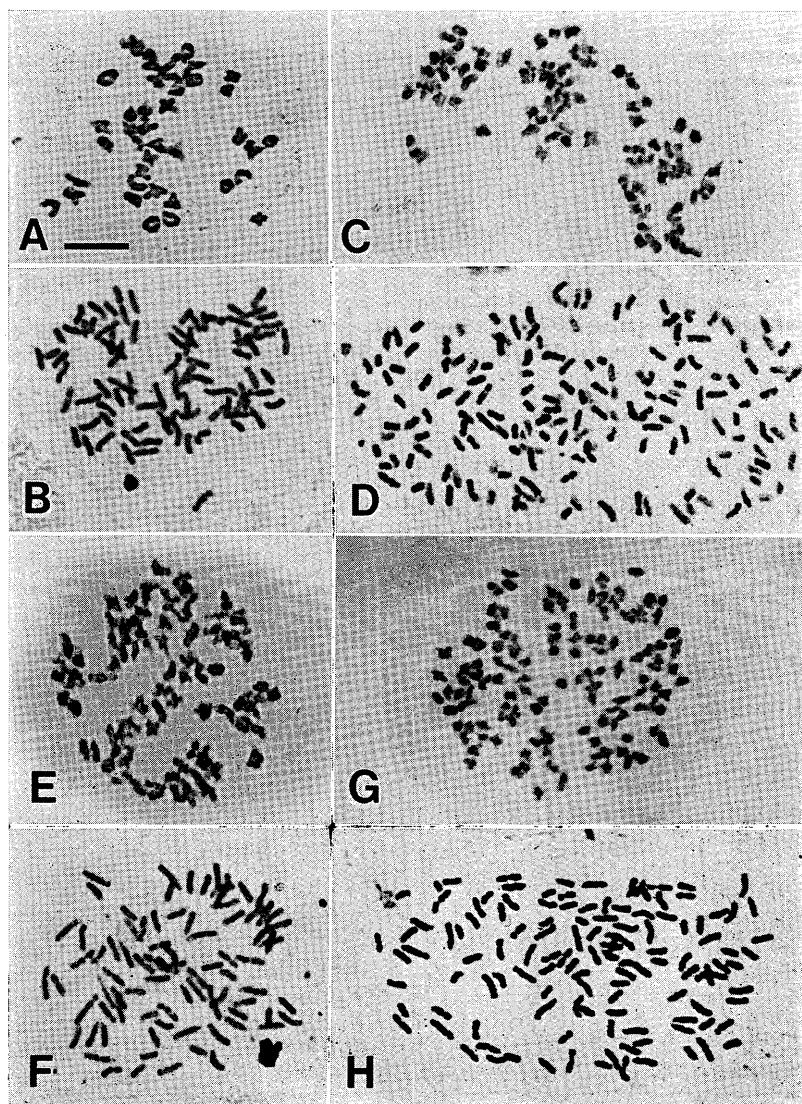


Fig. 1. Chromosome numbers of four types of *D. sparsa*. A, B. Sexual diploid with $n=41$ (A) and $2n=82$ (B), D. Salak-4. C, D. Sexual tetraploid with $n=82$ (C; Na. 4) and $2n=164$ (D; Han. 7). E, F. Agamosporous diploid with ' n '=82 (E; D. Ambo IV-4) and $2n=82$ (F; TY. 600-8). G, H. Agamosporous triploid with $n=123$ (G; Os. 3) and $2n=123$ (H; Os. 8). Bar=10 μ m.

(Fig. 2A, B) and an agamosporous triploid ($2n=123$). Of 10 plants examined, nine plants were sexual diploid and only one was agamosporous triploid. Only a sexual tetraploid ($n=82$ and/or $2n=164$) and a sexual diploid ($n=41$ and $2n=82$) were found in *D. rheophila* (Fig. 2C, D) and *D. angustipalea* (Fig. 2E, F), respectively.

The hybrids, *D. rheophila* × *D. sparsa* (Fig. 3A, B) was tetraploid ($2n=164$) and *D. sabaiei* × *D. sparsa* var. *sparsa* and *D. sabaiei* × *D. sparsa* var. *ryukyuensis* (Fig. 3C, D) were triploid ($2n=123$). The chromosome numbers of the hybrids

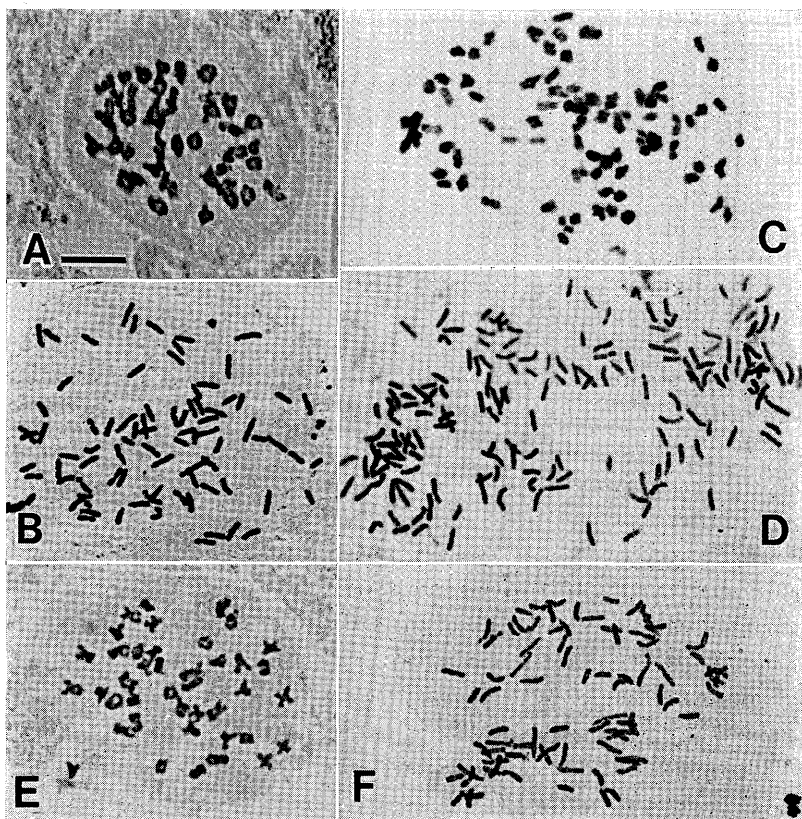


Fig. 2. Chromosome numbers of species related to *D. sparsa*. A, B. *D. melanocarpa* with $n=41$ (A; JM & TK.7) and $2n=82$ (B; TH & SP.1). C, D. *D. rheophila* with $n=82$ (C) and $2n=164$ (D), TY.52A. E, F. *D. angustipalea* with $n=41$ (E; JM & TK.7) and $2n=82$ (F; TH & SP.1). Bar=10 μ m.

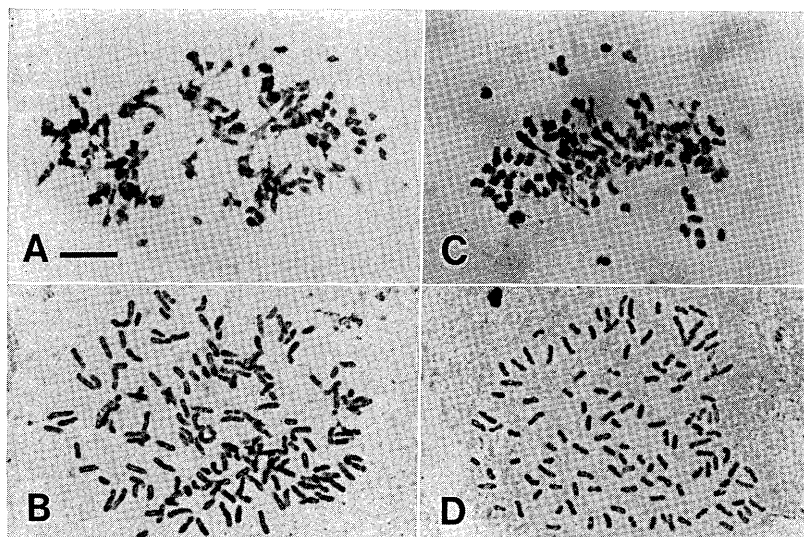


Fig. 3. Chromosome numbers of *D. rheophila* × *D. sparsa* with irregular meiosis (A) and $2n=164$ (B), TY.50B. *D. sabaei* × *D. sparsa* var. *ryukyuensis* with irregular meiosis (C) and $2n=123$ (D), D. 44a. Bar=10 μ m.

are, as expected, the sums of the gametic chromosome numbers of their parental species ($n=41$ of *D. sabaei*, $n=82$ of the tetraploid type of *D. sparsa*, and $n=82$ of *D. rheophila*). Meiosis in the hybrids was always so irregular that only abnormal-shaped, abortive spores were produced, which did not develop into any normal gametophytes in culture.

The reproductive modes assumed from the spore numbers are shown in Tab. 2. *Dryopteris sparsa* shows both sexual and agamosporous reproduction, although sexual plants are dominant and widely distributed over its range (Fig. 4). *Dryopteris melanocarpa* was sexual for all plants examined except for one agamosporous plant. *Dryopteris angustipalea*, *D. caccaina*, *D. indonesiana*, and *D. rheophila* are exclusively sexual, as far as known.

Discussion Our observations characterize both the cytology and reproductive mode of *D. sparsa* and its closely related species. They agree with previous studies that *D. sparsa* is cytologically polymorphic (Darnaedi & Iwatsuki 1987, Gibby 1985, Hirabayashi 1974, Kurita 1966, Manton 1955, Manton & Sledge 1954, Mehra 1961). Fig. 4 shows the geographic distribution of the

Tab. 2. Numbers of spores per sporangium and assumed mode of reproduction.

| Species | Number of specimens* | Spore number | Reproduction |
|------------------------|----------------------|--------------|--------------|
| <i>D. sparsa</i> | 133(38) | 64 | sexual |
| | 43(15) | 32 | agamosporous |
| <i>D. angustipalea</i> | 8(2) | 64 | sexual |
| <i>D. rheophila</i> | 17(12) | 64 | sexual |
| <i>D. melanocarpa</i> | 24(9) | 64 | sexual |
| | 2(1) | 32 | agamosporous |
| <i>D. caccaina</i> | 2(-) | 64 | sexual |
| <i>D. indonesiana</i> | 3(-) | 64 | sexual |

* Numbers in parentheses indicate numbers of specimens cytologically examined as well.

cyto-reproductive types of *D. sparsa*. The sexual tetraploid is the most widely distributed, while the sexual diploid occurs in the Himalayas, Sri Lanka, Malay Peninsula, and western Java, and probably elsewhere. The agamosporous diploid is confined to a narrow area of Yakushima Island, and the agamosporous triploid occurs in the Himalayas, Japan, and western Java. Compared with the agamosporous types, the sexual types are much more widely distributed, covering the entire range of the species.

Morphologically the agamosporous types of *D. sparsa* do not significantly differ from the sexual types (Darnaedi 1989). Electrophoretic data (Darnaedi 1989) indicates that the agamosporous triploid is an autopolyploid. An autopolyploid derivation of agamosporous races via unreduced spores was suggested for *Pellaea glabella* (Gastony 1988).

The variation in cytology and reproductive mode of *D. sparsa* seems to be related to the polymorphism in phenetic characters (Darnaedi 1989). Further studies in morphology, cytology, and reproductive biology are needed to clarify the taxonomic structure of *D. sparsa*.

It is noteworthy with special reference to the origin of *D. yakusilvicola* that the agamosporous diploid of *D. sparsa* occurs in Yakushima Island. *Dryopteris yakusilvicola*, an agamosporous triploid species endemic to Yakushima Island, is believed to have originated by hybridization between *D. sabaei* and

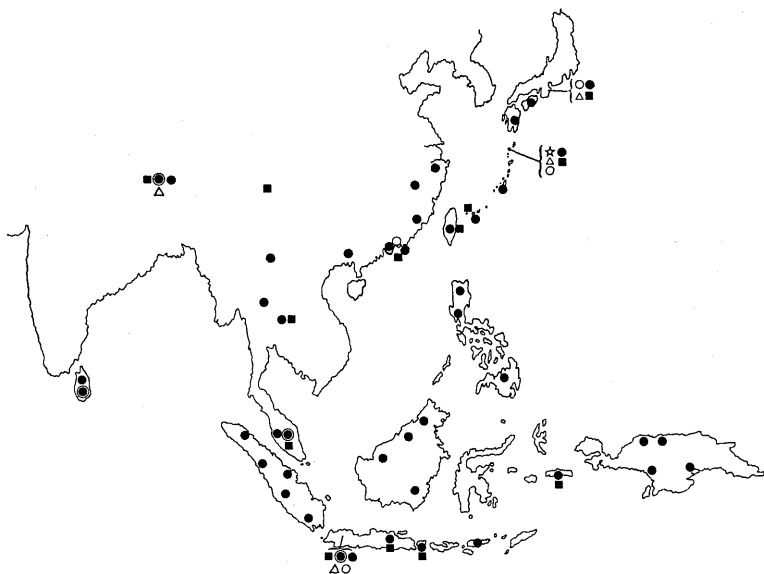


Fig. 4. Distribution of four cyto-reproductive types of *D. sparsa*, based on the results of this study, Darnaedi & Iwatsuki (1987), Gibby (1985), Hirabayashi (1974), Kurita (1966), Manton (1955), Manton & Sledge (1954), and Mehra (1961). The occurrence of symbols indicates general distribution pattern, and not the frequency of types. ●, sexual diploid; ☆, agamosporous diploid; ■, sexual tetraploid; △, agamosporous triploid; ◎, cytologically unknown sexual type; ○, cytologically unknown agamosporous type.

D. sparsa (Hirabayashi 1974, Mitui 1982, Darnaedi & Iwatsuki 1987). Two means of hybrid origin can be postulated: (1) *D. sabaei* × agamosporous diploid of *D. sparsa*; (2) *D. sabaei* × sexual tetraploid of *D. sparsa*. In the former case, which may be considered a simpler way, the agamosporous reproduction of *D. yakusilvicola* is derived from that of *D. sparsa*, whereas in the latter it would have to be newly acquired during the origin of *D. yakusilvicola*. Electrophoretic evidence supports the latter case (Darnaedi et al. submitted).

In contrast with *D. sparsa*, *D. angustipalea* and *D. rheophila* are uniform in their cytology and reproductive mode. They also exhibit small morphological variation and narrow distribution ranges (Darnaedi et al. 1989). *Dryopteris angustipalea* and *D. rheophila* seem to be local species, and the latter is ecologically specialized. They may be related to diploid and tetraploid types of *D. sparsa*, respectively.

Dryopteris rheophila is a facultative rheophyte (Yahara pers. comm.) and does not differ to any great degree from *D. sparsa* in morphological characters (Darnaedi et al. 1989). However, the irregular meiosis and abortive spores of their hybrid, *D. rheophila* × *D. sparsa*, indicate that *D. rheophila* is so genetically isolated as to warrant species separation. The sterile interspecific hybrids, *D. sabaei* × *D. sparsa* var. *sparsa* and *D. sabaei* × *D. sparsa* var. *ryukyuensis*, as well as *D. × rarissima* and *D. × yamashitae* (Hirabayashi 1974), present a similar taxonomic situation.

We are grateful to Drs. T. Yahara, J. Murata, and N. Murakami for valuable discussion and providing materials, and to Dr. C.M. Kuo, Messrs. N.K. Kabinawa, Gozali, K. Ohora, T. Kawahara, T. Suzuki, and T. Hasebe for providing materials. Our special thanks are due to Dr. M.G. Price for reading the manuscript. We are also indebted to Messrs. K. Hirai and A. Kabe who took care of living materials. We gratefully acknowledge the directors of BM, BO, KYO, and SING for loaning specimens for this study. This study was partly supported by a grant from Toray Science Foundation (to KI) and Grant-in-Aid, 61041020 (to MK), for Mombusho International Scientific Research Program.

References

- Darnaedi, D. 1989. Systematic study of *Dryopteris sparsa* complex (Dryopteridaceae). Dr. Sci. Thesis, Univ. Tokyo. ——— & K. Iwatsuki 1987. On the structure and systematic position of the fern *Dryopteris yakusilvicola* Kurata. J. Fac. Sci. Univ. Tokyo III, 14: 121-136. ———, M. Kato & K. Iwatsuki 1989. Five new or ill-defined species related to *Dryopteris sparsa* (Dryopteridaceae). Journ. Jap. Bot. 64: 299-310. ———, ——— & ———. Enzyme electrophoretic evidence for the origin of *Dryopteris yakusilvicola* (Dryopteridaceae). Bot. Mag. Tokyo (submitted). DeVol, C.E. & C.M. Kuo 1975. Dryopteridaceae. In H.L. Li et al. (eds.), Flora of Taiwan 1: 359-400. Epoch Publ., Taiwan. Gastony, G.J. 1988. The *Pellaea glabella* complex: electrophoretic evidence for the derivations of the agamosporous taxa and a revised taxonomy. Amer. Fern J. 78: 44-67. Gibby, M. 1985. Cytological observations on Indian subcontinent and Chinese *Dryopteris* and *Polystichum* (Pteridophyta: Dryopteridaceae). Bull. Brit. Mus. (Nat. Hist.) Bot. 14: 1-42. Hirabayashi, H. 1974. Cytogeographic study on *Dryopteris* of Japan. Harashobo,

Tokyo. Kurita, S. 1966. Chromosome numbers of some Japanese ferns 6. Journ. Jap. Bot. 44: 207-214. Lovis, J.D. 1977. Evolutionary patterns and processes in ferns. Advances in Botanical Research 4: 229-415. Manton, I. 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge Univ. Press, London. ——— 1955. Cytological notes on one hundred species of Malayan ferns. Appendix to R.E. Holttum, Rev. Fl. Malaya. Vol. 2. Ferns of Malaya. Governm. Print. Office, Singapore. ——— & W.A. Sledge 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. Phil. Trans. Roy. Soc. B. 238: 127-185. Mehra, S. 1961. Chromosome numbers in Himalayan ferns. Res. Bull. Panjab Univ. N.S., 12: 139-164. Mitui, K. 1982. Spore morphology of Japanese ferns. Hojoshokan, Tokyo. (in Japanese). Nakaike, T. 1975. Enumeratio Pteridophytarum Japonicarum. Filicales. Univ. Tokyo Press, Tokyo. Walker, T.G. 1979. The cytogenetics of ferns. In A.F. Dyer (ed.), The experimental biology of ferns, pp. 87-132. Academic Press, London.

* * * *

ナガバノイタチンダ, その近縁 5 種およびそれらの雑種の染色体数と生殖様式を明らかにするため細胞分類学的研究を行った。生殖様式については孢子囊あたりの孢子数による推定も合わせて行った。ヒマラヤから東・東南アジアに広く分布し、形態的にも多様なナガバノイタチンダには 2 倍体と 4 倍体の有性生殖型, 2 倍体と 3 倍体の無配生殖型の 4 型があった。そのうち 4 倍体有性生殖型が最も広く分布する。屋久島にのみ知られる 2 倍体無配生殖型は雑種起源と推定される 3 倍体無配生殖種コスギイタチンダの母種である可能性があったが、電気泳動研究によって否定された (ダルナエディ他 1989)。台湾に分布するクロミノイタチンダにはまれに 3 倍体無配生殖型もあるが、大部分は 2 倍体有性生殖型である。*D. angustipalea* と *D. rheophila* は、それぞれ 2 倍体と 4 倍体の有性生殖種である。*D. rheophila* はタイ東北部に分布する 条件的溪流沿い植物である。本種とタイに分布する 4 倍体有性生殖型ナガバノイタチンダの形態的な違いは大きくないが、それらの間の雑種 (4 倍体) は減数分裂は異常で正常な孢子はできない。したがって *D. rheophila* はナガバノイタチンダとは別種である。*D. caccina* (ホウライイタチンダ) と *D. indonesiana* は染色体数は不明であるが有性生殖種である。